REMARKS

Reconsideration and allowance are respectfully requested.

Claims 1-13 and 24 are pending. Non-elected claims 14-23 were withdrawn from consideration by the Examiner. No new matter has been added by amending the claims because the original disclosure supports the claim amendments.

The Examiner has requested clarification of Applicants' argument against the lack of unity requirement. Applicants confirm that they never stated that the individual species of Schiff base forming compounds were not distinct inventions. But the statement on page 3 of the Action ("if Applicants regard each compound can constitute a distinct invention from the other, the unity of invention would have been broken") is disputed. The PCT Articles and Rules only require that a "special technical feature" be shared by the claims for them to be examined in the same application. The claims can, in addition, be separately patentable without breaking unity of invention. The Examiner is respectfully requested to cite legal authority for the statement quoted above.

35 U.S.C. 112 – Definiteness

Claims 2-6 and 11 were rejected under Section 112, second paragraph, as being allegedly "indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." Applicants traverse.

The terms "substantially" and "about" have been deleted; it has been clarified that both administration of the compound and the nucleotide sequence are repeated in claim 6. Applicants request withdrawal of the Section 112, second paragraph, rejection because the pending claims are clear and definite.

35 U.S.C. 103 – Nonobviousness

A determination of *prima facie* obviousness requires a reasonable expectation of success. See *In re Rinehart*, 189 USPQ 143, 148 (C.C.P.A. 1976).

Claims 1-4, 7-9, 11-13 and 24 were rejected under Section 103(a) as allegedly being unpatentable over Rhodes (U.S. Patent 5,508310) in view of Herrmann et al. (U.S. Patent 5,620,896). Claim 10 was rejected under Section 103(a) as allegedly being

unpatentable over Rhodes in view of Herrmann et al. and Bellhouse et al. (U.S. Patent 5,630,796). Applicants traverse both rejections.

On page 4 of the Action, the Examiner agrees that Rhodes excludes tucaresol as a Schiff base forming compound for potentiation of the immune response but she maintains, "Rhodes does not exclude tucaresol as a Schiff base forming compound and an immunopotentiator." With regard to the rejection of claims 1-4, 7-9, 11-13 and 24 as not patentable in light of Rhodes and Herrmann et al., Applicants can refute absolutely the assertion that the mechanism of tucaresol's mechanism of action is the same regardless of whether it is used as an adjuvant for a conventional protein vaccine or a DNA vaccine according to Applicants' invention. These two mechanisms are not the same. Moreover, at the time the invention was made, there were good reasons to have believed that mechanistically tucaresol would not work in the setting of a DNA vaccine.

The Examiner has stated that our previous arguments were not deemed persuasive, "In fact, Rhodes demonstrates that tucaresol increased T-lymphocyte priming to antigen . . . ; therefore, Rhodes teaches tucaresol as an immunopotentiator and an effective vaccine adjuvant" (page 4 of the Action). This interpretation of Rhodes is not based on the reference's disclosure but, instead, uses hindsight gained from Applicants' teaching of the efficacy of tucaresol in DNA vaccination. Such use of hindsight is not a proper basis for establishing a case of prima facie obviousness.

Rhodes defined **immunopotentiator** as "an agent which is capable of restoring a depressed immune function, or enhancing normal immune function, or both" (column 1, lines 52-57). Subsequently, **immune function** was defined as "the development and expression of humoral (antibody-mediated) immunity, cellular (T-cell-mediated) immunity, or macrophage and granulocyte mediated resistance" (column 5, lines 24-28). This term was being used in the context of so-called wild-type infections and other disease settings such as malignancy, where the antigens to which the immune response is to be enhanced are provided by tumors in and infections of the body (see column 1, lines 7-12). Rhodes also provides for the use of tucaresol as an adjuvant for a protein vaccine (see column 14, lines 33-37). He suggests that a vaccine may be prepared by formulating the antigenic component with the compound. Rhodes used tucaresol in a setting

in which the protein antigens to which the immune response was to be enhanced would be co-administered with the adjuvant, i.e., as a conventional protein vaccine.

Neither Rhodes nor Herrmann et al. was contemplating the use of tucaresol (or any other Schiff base forming compound) to enhance the immune response to a DNA vaccine. For the reasons set out below, the two cases are quite different. In both cases (immunopotentiation and vaccine adjuvancy), tucaresol was shown to be exerting its effects by amplifying co-stimulatory or 2nd signals of the immune response. The mechanism of handling both wild-type antigens contributed by an infection and conventional vaccine antigens (see ref. 1 of Appendix II) differs from the way DNA-encoded antigens are handled in a number of fundamental ways. In an infection, antigens are generated by: pathway A in which the synthetic machinery of the host cell when it is taken over by the virus for the production of viral proteins (see attached Fig. A of Appendix I) or pathway B in which whole micro-organisms or fragments of micro-organisms taken up from the inter-cellular environment by phagocytic cells (see attached Fig. B of Appendix I).

The same two routes operate for conventional vaccines in which pathway A predominates for live attenuated vaccines and pathway B predominates for killed and sub-unit vaccines. Importantly, both pathways provide an array of danger signals and co-stimulatory signals initiated by pathogen associated molecular patterns (PAMPs) during the uptake/entry phase into APC (refs. 2-4 of Appendix II).

In contrast, antigens provided by DNA encoded vaccines are taken up in a different way and utilize a unique mechanism of antigen handling that involves neither of these pathways. Importantly, DNA vaccines do not contribute the array of danger/co-stimulatory signals initiated by PAMPs and other microbial elements. This is illustrated in Fig. C of Appendix II which shows that plasmid DNA directly transfects the cell and lacks the microbial elements that amplify co-stimulation (ref. 5 of Appendix II).

Subsequent events in terms of binding to MHC molecules and ligating the T-cell receptor are the same for all three kinds of delivery (ref. 1 of Appendix II). However, the important difference is in the co-stimulatory environment which is absent in DNA vaccination as shown in pathway C (see attached Fig. C of Appendix II). Adjuvants exert their effects on the co-stimulatory environment (ref. 6 of Appendix II) and both conventional

adjuvants and tucaresol are effective in pathways A and B. It is therefore surprising that tucaresol was found to work in the case of DNA vaccination (i.e., pathway C) where the co-stimulatory environment is absent or very weak.

Adjuvants work on co-stimulatory mechanisms and do not affect the recognition of antigen by the T-cell receptor or the signal it transduces (refs. 6-7 of Appendix II and see Fig. D of Appendix I). Instead, they work through ancillary receptors such as toll-like receptors to amplify co-stimulation of (ref. 8 of Appendix II). Accessory/co-stimulatory signals are known to be interdependent and integrated at a number of levels within antigen presenting cells and T cells (ref. 9 of Appendix II). Tucaresol also exerts its effects on co-stimulation (ref. 10 of Appendix II). Because the co-stimulatory environment associated with conventional protein vaccines (and natural infections) is very different from the co-stimulatory environment associated with DNA vaccination, there would not be a reasonable expectation of success in using tucaresol with DNA vaccines and DNA immunization.

During 1995-96, large animal studies were indicating that adjuvants were likely to be needed for DNA vaccination and there was an expectation that, because of the fundamental differences between DNA vaccination and other forms of vaccination (e.g., using protein vaccines), adjuvants that worked for conventional protein vaccines would be unlikely to work for DNA vaccines (ref. 8 of Appendix II). It is for this reason that other approaches such as co-administering cytokines were pursued rather than adjuvants previously used for protein vaccines such as tucaresol (ref. 9 of Appendix II).

Another surprising feature of Applicants' claimed invention is the demonstration of amplification of the IgG1 subclass of antibody to mycobacterial HSP-65 by tucaresol when used in conjunction with a DNA vaccine. All previous observations of tucaresol have indicated that it switches immune responses to the Th1 type of response, amplifying Th1 cytokines such as IFN-γ but not Th2 cytokines such as IL-4 (ref. 9 of Appendix II). However the IgG1 subtype is a Th2 subtype of antibody in the mouse.

Furthermore Applicants reiterate their comments made in the last response to the effect that known immunopotentiating agents have been tried in combination with DNA vaccines (as disclosed on page 3, lines 27-35, of the specification) with limited or mixed

success and the conventional adjuvants such as alum, FCA, and FIA are not effective as adjuvants in DNA vaccination, as demonstrated in Example 1 of Applicants' specfication. A person of skill in the art would thus not have had a reasonable of success to assume that adjuvants successfully used in conventional protein vaccination would also be effective as adjuvants in DNA vaccination. It was all the more surprising therefore that Schiff base forming compounds such as tucaresol, an effective conventional vaccine adjuvant, could be successfully used in a DNA vaccine setting. The Examiner is therefore incorrect in citing Rhodes as providing a disclosure that alleges the usefulness of tucaresol in a DNA vaccine setting or even that there would be any motivation to use tucerasol in such a setting with any expectation of success.

The other reference cited by the Examiner, Herrmann et al., does not disclose any particular adjuvants or classes of adjuvants which might be expected to work, and provides no working examples using any such adjuvant. Moreover, the purpose of the adjuvant described by Herrmann et al. is to "promote DNA uptake" or "recruitment of immune system cells to the site." These might be termed adjuvants by Herrmann et al., but they are not the same as agents which would "enhance both humoral and cellular immune responses initiated by the antigenic peptide" as required by the claims of the present invention. This establishes that the term "adjuvant" is used to serve completely different purposes by Rhodes and Herrmann et al. The latter reference would not therefore provide any additional motivation to that disclosed in Rhodes, and in view of the lack of success reported with conventional adjuvants in enhancing the immune system's response to DNA vaccines, no expectation of success can be derived from the disclosure by Herrmann et al. of "adjuvants" which are intended to serve a completely different function.

The combined teachings of Rhodes and Herrmann et al. would not therefore suggest to a skilled artisan that Schiff base forming compounds such as tucaresol, will achieve enhancement of the immune response in a DNA vaccine setting, where other conventional adjuvants will not, nor that such compounds will achieve this utility by enhancing both the humoral and cellular immune responses initiated by the antigenic peptide expressed by the nucleotide sequence which forms the DNA vaccine. The

Bellhouse et al. reference was cited for its disclosure of a gene gun, which does not remedy the defects noted above with respect to Rhodes and Herrmann et al.

Withdrawal of the Section 103 rejection is requested because the invention as claimed would not have been obvious to a person of ordinary skill in the art at the time it was made.

Conclusion

Having fully responded to all of the pending objections and rejections contained in the Office Action (Paper No. 11), Applicants submit that the claims are in condition for allowance and earnestly solicit an early Notice to that effect. The Examiner is invited to contact the undersigned if any further information is required.

Respectfully submitted,

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APPENDIX
MARKED-UP VERSION TO SHOW CHANGES

IN THE CLAIMS

The claims are amended as follows.

2. (Amended) The method according to claim 1 wherein administration of the compound takes place on between one and seven occasions. between [about] 14 days prior to and [about] 14 days post administration of the nucleotide sequence.

3. (Amended) The method according to claim 1 wherein administration of the compound takes place on between one and seven occasions, between [about] 7 days prior to and [about] 7 days post administration of the nucleotide sequence.

4. (Amended) The method according to claim 1 wherein administration of the compound takes place between [about] 24 hours prior to and [about] 24 hours post administration of the nucleotide sequence.

5. (Amended) The method according to claim 1 wherein administration of the compound is [substantially] simultaneous with administration of the nucleotide sequence.

6. (Amended) The method according to any one of claims 1-5 wherein administration of the compound and the nucleotide sequence [claim 1 which] is repeated between 1 and 4 times, at intervals of between [about] 1 day and [about] 18 months.

7. (Amended) The method according to claim 1 wherein administration of the nucleotide sequence is via the oral, nasal, pulmonary, intramuscular, subcutaneous or intradermal route[s].

9. (Amended) The method according to claim 1 wherein administration of the compound is via the oral, nasal, pulmonary, intramuscular, subcutaneous, intradermal or topical route[s].

11. (Amended) The method according to claim 9 wherein the compound is administered at a dose of between [about] 0.1 mg/kg and [about] 100 mg/[per]kg per administration.